## REMARKS/ARGUMENTS

Applicants respectfully request reconsideration and allowance of this application in view of the amendments above and the following comments.

Main claim 12 has been replaced by new claim 34, which is modeled on claim 33, but in step (a) tracks the "isolating a population of $CD4^+$ $CD25^+$ T cells" language that the Examiner indicated on page 2 of the final rejection was part of the concededly enabled method. (Applicants deleted the "a population of" language as done previously with respect to claim 33; see the amendment filed June 16, 2010, and the explanation in the first paragraph on page 10 thereof.) The instant specification clearly supports the new wording, for example, at page 10, lines 8 ff ("blood is flowing through columns and $CD4^+$ $CD25^+$ T cells ***are removed*** ***"); and page 18, lines 24-28 ("$CD4^+$ $CD25^+$ T cells ***were isolated*** from the pure [$CD4^+$ T cells isolated from PBMC]."

Claim 33 has been amended in step (c) to require that the constitutive expression of CTLA-4 in the $CD4^+$ $CD25^+$ regulatory T cells be confirmed, not merely tested for. New claim 34 contains the identical requirement in step (b). This requirement finds clear support in the specification, for example, in the sentence bridging pages 3-4; in the sentence bridging pages 11-12; and at page 21, lines 11-13.

New claims 35 and 36 are supported by, for example, page 14, lines 29-32, indicating the *ex vivo* $CD4^+CD25^+$ T cells were stained with anti-CTLA-4 antibodies; and page 21, lines 11-14, confirming the $CD4^+$ $CD25^+$ regulatory T cells constitutively express CTLA-4.

The remaining amendments to the claims are purely editorial.

Applicants do not believe that any of the amendments introduce new matter. An early notice to that effect is earnestly solicited.

Claims 12 and 25-32 were rejected under 35 USC § 112, first paragraph, as being broader than the enabling disclosure. In response, Applicants respectfully submit that the cancellation of claim 12 moots this rejection.

As noted above, new main claim 34 tracks the language of claim 33, which was not subject to this rejection. Indeed, the last step of claim 34 is identical to the last step of claim 33; and the first step of claim 34 is similar in wording to the first step of claim 33, but is a bit broader, again, partially tracking the language that the Examiner indicated was enabled in the middle of page 2 of the final rejection.

In short, Applicants do not believe that new main claim 34 should be subject to this or a similar lack of enablement rejection. Since claims 25-32 have been made dependent on claim 34, likewise, claims 25-32 should also be free of this or a similar lack of enablement rejection. An early notice that all claims are enabled is earnestly solicited.

Claim 33 was rejected under 35 USC § 103(a) as being obvious over Koulis et al. ("Koulis"), *J. Allergy Clin. Immunol.*, S294-S295 (2001), in view of Read et al. ("Read"), *J. Exp. Med., 192:* 295-302 (2000), and Leung et al. ("Leung"), *J. Biol. Chem., 270:* 25107-25114 (1995). In response, Applicants respectfully renew their argument that the cited combination of references fails to make out a *prima facie* case of the obviousness of claim 33.

First, at the top of page 7 of the final rejection, the Examiner notes that previous claim 33 merely required testing for CTLA-4 expression, not CTLA-4 detection, and persons skilled in the art would have had a reasonable expectation of success in merely testing for CTLA-4. In response, as noted above, claim 33 has been amended to require detecting CTLA-4 ("confirming the presence in said $CD4^+$ $CD25^+$ T cells of $CD4^+$ $CD25^+$ regulatory T cells constitutively expressing CTLA-4").

Second, the Examiner finds Koulis' method of isolating PBMC from blood technically meets the terms of a method requiring isolating $CD4^+$ T cells from blood. Applicants respectfully disagree. The result of Koulis' method is not isolated $CD4^+$ T cells, but, rather, isolated PBMC.

Third, as previously argued in the second paragraph on page 10 of the amendment filed June 16, 2010, in the cited combination of references, there is no teaching or suggestion to isolate $CD4^+$ T cells, and then to isolate $CD4^+CD25^+$ T cells from the isolated $CD4^+$ T cells. In other words, the cited combination of references does not teach or suggest carrying out ***both*** instant steps (a) and (b). Consequently, the cited combination of references fails to make out a *prima facie* case of the obviousness of claim 33 for this reason alone. In the final rejection, the Examiner does not respond to this previous argument.

Fourth, Applicants also respectfully disagree that Read would have provided any person having ordinary skill in the art with the reasonable ***expectation*** that human blood should contain $CD4^+$ $CD25^+$ regulatory T cells that constitutively express CTLA-4. Applicants respectfully submit that an "expectation" of something is quite a bit more than the "mere possibility" of that

thing. At best, the Examiner's reasoning establishes only the mere possibility that human blood might contain $CD4^+$ $CD25^+$ regulatory T cells that constitutively express CTLA-4; the Examiner does not give a single reason why persons skilled in the art should ***expect*** human blood contains $CD4^+$ $CD25^+$ regulatory T cells that constitutively express CTLA-4.

According to the Examiner, it is well established that mice are suitable models for studying the human immune system, and are very similar in terms of T cell phenotype and function. Even if true, there remain significant differences between human and murine cells and proteins, and it is not the case that, given any similarities, the ordinary artisan would have had a reasonable expectation that human regulatory T cells would express CTLA-4 in a similar manner to mice.

Indeed, the existence of a cell surface molecule on a certain cell type in mice does not at all lead to an expectation that such a molecule is definitely present on the corresponding human cells, if any. Differences between the immune system of humans and mice are well known, although admittedly not always appreciated, which is one reason why humans can often heal mice but not themselves [See: "Of mice and not men: differences between mouse and human immunology," J. Mestas, et al., *J. Immunol., 172(5):* 2731-8 (2004), copy enclosed.]

A large number of differences between mice and humans are already well documented, including differences in glycoprotein, ligand, and receptor expression; and in development and function of both B cells and T cells. For example, the Ly49 family of proteins, which is expressed on NK and NKT cells in mice, is ***completely absent*** in humans, which use, instead, KIR family proteins as NK inhibitory receptors; see in Mestas the fourth paragraph in the left-

hand column on page 2732. NK cells (natural killer cells) in mice carry NK receptors of the ***lectin type*** while in humans the receptors are of the ***immunoglobulin type!*** Mouse B cells express one, but never both of CD5 and CD23, whereas human B cells always express both; see in Mestas the fourth paragraph in the right-hand column on page 2732. Human B cells express CD38, whereas mouse B cells ***do*** *not;* see in Mestas also the fourth paragraph in the right-hand column on page 2732. Murine peripheral T cells express Thy-1, whereas, in humans, Thy-1 is only expressed ***on neurons****;* see in Mestas the last paragraph in the right-hand column on page 2732. IL-13 induces switching to IgE in humans, but has ***no effect*** whatsoever on mouse B cells; see in Mestas the second paragraph in the right-hand column on page 2732. These are only a few examples—many more such differences between mice and man are discussed in Mestas.

Respectfully, the significant differences between mice and humans are such that there could not have been a reasonable expectation, at the time the present invention was made, that because a glycoprotein is expressed in mice and has a particular function, the counterpart glycoprotein must either exist in humans and/or perform the corresponding function in humans. As Mestas and his colleagues note:

> "Despite [relative gene] conservation [between the species] there exist significant differences between mice and humans in immune system development, activation, and response to challenge, in both the innate and adaptive arms. * * * [T]here has been a tendency to ignore differences and in many cases, perhaps, make the assumption that what is true in mice—in vivo veritas—is necessarily true in humans."

See in Mestas the paragraph bridging the two columns on page 2731.

Respectfully, the existence of the human $CD4^+$ $CD25^+$ regulatory T cells could not even have been presumed given the knowledge of the existence of the murine counterparts, let alone that the human $CD4^+$ $CD25^+$ regulatory T cells would constitutively express CTLA-4. As noted in the specification, although it was evident for years that the $CD4^+CD25^+$ T cell population in mice constituted natural regulatory T cells (nTregs), it was, however, with the passage of time becoming increasingly less likely that $CD4^+$ T cells exhibiting similar properties naturally occurred in humans. Thus, the nTregs in humans were discovered many years after their murine counterparts despite the enormous interest in these new "suppressor cells." In fact, the art previously believed that the human $CD4^+$ $CD25^+$ regulatory T cells actually represented conventional ***memory cells*** rather than suppressive cells as had been published. Importantly, it was shown that the $CD4^+CD25^+$ T cells were ***not*** anergic as would have been absolutely typical for nTreg. This shows how far afield the art was on this significant point. A person having ordinary skill in the art, given Koulis and Read, would not, at the time the present invention was made, have been motivated to carry out the method of claim 33 with a reasonable expectation of successfully confirming the existence in human blood of $CD4^+$ $CD25^+$ regulatory T cells constitutively expressing CTLA-4.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw this rejection. An early notice that this rejection has been reconsidered and withdrawn is earnestly solicited.

Applicants believe that the foregoing constitutes a bona fide response to all outstanding objections and rejections.

Applicants also believe that this application is in condition for immediate allowance. However, should any issue(s) of a minor nature remain, the Examiner is respectfully requested to telephone the undersigned at telephone number (212) 808-0700 so that the issue(s) might be promptly resolved.

Early and favorable action is earnestly solicited.

Respectfully submitted,

NORRIS MCLAUGHLIN & MARCUS, P.A.

By /Kurt G. Briscoe/

Kurt G. Briscoe
Attorney for Applicant(s)
Reg. No. 33,141
875 Third Avenue - 8th Floor
New York, New York 10022
Phone: (212) 808-0700
Fax: (212) 808-0844